REMARKS

Favorable reconsideration of the subject application is respectfully requested in view of the following remarks. Claims 30-34, 73 and 74 are pending and under consideration. By this amendment, claim 30 is amended to more specifically recite one aspect of the invention. Support for the amendment is provided throughout the specification and claims as originally filed, and the amendment does not constitute new matter. Specific support for peptide analogues wherein the N-terminal and/or C-terminal amino acid is a D-amino acid is provided, *e.g.,* on page 3, lines 16-17. This amendment is not to be construed as acquiescence to any rejection and is made without prejudice to prosecution of any subject matter modified by the amendment in a related divisional, continuation, or continuation-in-part application.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 30-34 stand rejected under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite in reciting alterations of the N- and/or C-terminal amino acid residues of the claimed peptides. Specifically, the Examiner asserts that the claimed peptides may comprise sequence in addition to the natural MBP sequence. Therefore, the identity of the N- and/or C-terminal amino acids being altered is unclear, and, consequently, the substitutions constituting an alteration are unclear.

Applicants respectfully traverse this basis of rejection and submit that the skilled artisan would readily discern the nature of an N- and/or C-terminal amino acid alternation, in light of the teachings of the specification. However, to expedite prosecution of the instant application, and without acquiescence to this basis of rejection, claim 1 has been amended to specifically recite that the N- and/or C-terminal amino acids have been altered to D-amino acids. Applicants submit that the skilled artisan would readily understand that these alterations encompass any and all D-amino acids (none of which are naturally occurring amino acids) and, therefore, the claims satisfy the requirements of Section 112, second paragraph. Applicants respectfully request the Examiner reconsider and withdraw this basis of rejection in light of this amendment.

Rejection Under 35 U.S.C. § 103

Claims 31, 32, and 34 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Wucherpfenig *et al.* in view of Nishimote *et al.* The Examiner asserts that Wucherpfeneg *et al.* teaches peptide analogs comprising residues 84-102 of MBP with Ala or Arg substitutions of Lys 93, which read on the claimed peptides having Lys-91 substituted with Ala or Arg. The Examiner concedes that Wucherpfenig *et al.* fails to teach an MBP peptide analog having N- and/or C-terminal residues altered to reduce proteolysis. However, the Examiner asserts that Nishimoto *et al.* remedies this deficiency by teaching that the replacement of an L-amino acid residue with the corresponding D-isomer is a standard way of rendering a polypeptide less sensitive to proteolysis, and it would, therefore, be obvious to the skilled artisan to alter the terminal amino acid residues of the MBP peptide taught by Wucherpfenig *et al.* as described by Nishimoto *et al.* to incorporate a D-amino acid, in order to increase stability of the analog.

Applicants respectfully traverse this basis of rejection and submit that the Action fails to establish a *prima facie* case of obviousness in light of the combination of Wucherpfenig *et al.* and Nishimoto *et al.* More specifically, the Examiner fails to establish any motivation to combine the cited references to achieve the claimed invention. As established by the courts and enunciated in the M.P.E.P., "[o]bviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention when there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." M.P.E.P., 8th Ed. § 2143.01.

In the present case, neither reference teaches or suggests combining the references to achieve the claimed invention. In addition, neither reference would motivate the skilled artisan to combine their teachings to produce a claimed MBP peptide analogue wherein the N- and/or C-terminal residue is altered to a D-amino acid, since the understanding in the art was that substitutions from L- to D-isomers could not be assumed beneficial, as acknowledged by the Examiner in the Office Action mailed December 2, 2003 (page 5, lines 1-5). Indeed, the Examiner himself points to references that teach that substitution of L- to D-isomers may not be

beneficial for the inhibitory effect of a peptide analog, and, on the contrary, it may actually abolish the inhibitory effect (Teitelbaum *et al., Proc. Natl. Acad. Sci. USA* 85:9724-9728 (1988)). Accordingly, no motivation to combine the cited references has been established.

Applicants again emphasize that the mere fact that the teachings of the prior art *can* be combined or modified, or that a person having ordinary skill in the art is *capable* of combining or modifying the teachings of the prior art, does not make the resultant combination *prima facie* obvious, as the prior art must also suggest the desirability of the combination (*see, e.g., In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990); *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992)). Since neither of the cited references teach or suggest any advantage or desirability of modifying the teachings of the references to produce the claimed peptide analogue, Applicants submit that the Action fails to establish a *prima facie* case of obviousness.

Furthermore, Applicants note that the Federal Circuit has repeatedly emphasized that "obvious to try" is not the standard under Section 103. *See, e.g., In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1532 (Fed.Cir.1988). Rather, the Court has explained that whereas it may appear obvious to try an invention, this does not necessarily mean that the invention itself is obvious under Section 103. Rather, there are several types of instances where what may be obvious to try is not obvious under Section 103, including the situation where it might be considered obvious to explore a new technology or general approach that seemed to be a promising field where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *Id.*

Applicants submit that the instant situation is analogous to that described above, in that the cited prior art provides only general teachings regarding introducing a D-amino acid substitution into a peptide, but provides no specific guidance regarding how to make such a substitution in an MBP peptide analogue to achieve a peptide analogue subject to reduced proteolysis, as claimed. Accordingly, the present basis of rejection can at most be considered based upon an impermissible obvious to try standard, particularly in light of the knowledge in the art, discussed above, that such substitutions may negatively affect the functional properties of the peptide.

In light of these remarks, Applicants respectfully submit that the Action fails to establish a *prima facie* case of obviousness, since it does not demonstrate the cited references would provide motivation to combine their teachings to reach the presently claimed invention. Applicants, therefore, request that this basis of rejection be reconsidered and withdrawn.

Furthermore, Applicants submit that Wucherpfenig *et al.* is not valid prior art under Section 102(a), since Applicants possessed the MBP peptide analogues allegedly taught by Wucherpfenig *et al.* prior to the publication of Wucherpfenig *et al.*, which occurred within one year of the filing of the instant application. Applicants submit herewith a copy of two associated Declarations under 35 U.S.C. §1.131, which were previously filed in related U.S. Patent Application Serial No. 08/953,937, conclusively establishing that MBP peptides having the L-lysine at position 91 altered to another amino acid were conceived by Applicants prior to the publication of Wucherpfenig *et al.* and subsequently reduced to practice by Applicants. Applicants further submit that Nishimoto *et al.* on its own does not suggest the present invention, since it does not teach a claimed MBP peptide analog. Accordingly, Applicants respectfully submit that the cited references fail to anticipate the claimed invention, either alone or in combination, and request that this basis of rejection be withdrawn.

Double Patenting Rejection

Claims 30-34, 73 and 74 stand rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1, 2 and 7 of U.S. Patent No. 6,329,499 ("the '499 patent"). The Examiner asserts that while the subject matter of the instant claims is not identical to the subject matter claimed in the '499 patent, it is not patentably distinct, since the '499 patent claims peptide with substituted Lys-91 and optionally N- or C-terminal residues.

Applicants traverse this basis of rejection and submit that the subject matter of the present claims is patentably distinct from and non-obvious over the subject matter claimed in the '499 patent. However, in order to expedite prosecution of the instant application, Applicants have provided herewith a terminal disclaimer related to the '499 patent. Therefore, Applicants respectfully request that this basis of rejection be withdrawn.

The Director is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

Applicants respectfully submit that all of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

SEED Intellectual Property Law Group PLLC

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PATENT

I hereby certify that on the date specified below, this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, DC 20231.

September 14, 2000 Rozell A. Price

Date Rozell A. Price

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Lawrence Steinman et al.
Application No.	:	08/953,937
Filed	:	October 20, 1997
For	:	METHODS FOR TREATMENT OF MULTIPLE SCLEROSIS USING PEPTIDE ANALOGUES AT POSITION 91 OF HUMAN MYELIN BASIC PROTEIN
Examiner	:	Jeffrey E. Russel
Art Unit	:	1654
Docket No.	:	690068.412C1
Date	:	July 1, 2000

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF LAWRENCE STEINMAN, NICHOLAS LING AND PAUL J. CONLON UNDER 37 C.F.R. § 1.131

Sir:

Lawrence Steinman, Nicholas Ling and Paul J. Conlon do hereby declare that:

1. We are the coinventors of the subject matter in the above-identified patent application.

2. I, Lawrence Steinman, am presently employed by Stanford University, an assignee of the above-identified patent application. We, Nicholas Ling and Paul J. Conlon are presently employed at Neurocrine Biosciences, Inc., an assignee of the above-identified patent application.

3. We have reviewed the Examiner's Office Action dated October 29, 1999 (Paper No. 32), especially the § 102 and § 103 rejections: claims 1, 3, 5, and 6 under 35 U.S.C. § 102(a) as being anticipated by Wucherpfennig et al.; claims 1-3, 5, and 6 under 35 U.S.C. § 102(a) as being anticipated by Vogt et al.; and claims 6-9 and 11 under 35 U.S.C. § 103 as being unpatentable over Sharma et al. in view of Wucherpfennig et al. or Vogt et al. In order to assist the Examiner's evaluation of the application, we have provided the following remarks showing that the present invention was conceived of prior to the publication of Wucherpfennig (the earlier date) and diligently reduced to practice, culminating in the filing of the subject application.

4. We have reviewed our laboratory records and submitted proposals, including the Exhibits submitted herewith, and readily conclude that the peptides recited in claims 1-3, 5 and 6 were conceived prior to January 1994 (the publication date of Wucherpfennig et al.) or August 11, 1994 (the publication date of Vogt et al.). Further, the invention of claims 1-3, and 5 were actually reduced to practice prior January 1994. In addition, the methods recited in claims 6-9 and 11 were conceived of prior to January 1994, and were diligently pursued up to the filing of the present application.

5. With regard to claims 1 and 2, Exhibit 1 is a laboratory page recorded prior to January 1994, showing conception of the sequences of MBP 87-99 analogues[1]. In particular, the analogue that has a K>A substitution at residue 91 is shown. (In Exhibit 1, this residue is labeled 93, consensus of the numbering of human MBP was reached later in time). In accordance with the teachings of the specification on pages 5-6 and Example 1, in these experiments, peptides of human myelin basic protein containing residues 87-99 were

[1] Dates originally recorded on the laboratory pages and grant proposals have been redacted. The time frame relative to the publication dates of the cited art is presented instead.

synthesized. The wild type peptide sequence and peptides with single alanine substitutions were synthesized by an automated procedure on a peptide synthesizer (model 5050; Milli Gen, Burlington, MA) using standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by HPLC; the structure was confirmed by amino acid analysis and mass spectroscopy. The sequence of the wild-type peptide, called 89.0, is shown at the top of Exhibit 1. The peptide analogue with a position 91 alanine substitution (position 93 in Exhibit 1) is labeled peptide 89.5. Thus, an analogue was made with a 91 substitution, in particular, from lysine to the non-conservative amino acid alanine. Furthermore, as shown in Exhibit 2, the 91K>A peptide was tested in a proliferation assay, such as the one described on page 7, lines 13-24 and Example 6 of the specification. The peptide analogue, 91K>A (labeled p89.5 in Exhibit 2) failed to stimulate proliferation of an MBP-reactive T cell line at a concentration range of 10-100 μM.

6. With regard to other peptide substitutions at position 91 as recited in claim 3, Exhibit 3, dated prior to January 1994, is a page from a laboratory notebook showing peptide analogues in which position 91 is altered to alanine, leucine, valine, arginine, glutamic acid, asparagine, tyrosine, serine and aspartic acid.

7. As recited in claim 5, position 91 peptide analogues reduce production of TNF-α from MBP-reactive T cells. This concept was proposed in an application which was submitted to a granting agency prior to January 1994. In this proposal, one aim was specifically directed toward determining cytokine profiles of T cell clones that are reactive to MBP after incubation with native or mutated MBP peptides (Exhibit 4). Experiments were subsequently performed to examine the expression levels of TNF-α upon exposure to position 91 analogues in particular. Briefly, as similarly described in Example 11, draining lymph node cells from rats co-immunized with MBP 87-99 and MBP 87-99 [91K→A] were stimulated *in vitro* (10^7 cells/ml) with different concentration of peptides. After 12, 24, 48, and 72 hr of stimulation, supernatants were collected for cytokine detection. TNF-α expression levels were determined after 8 hr by a rat TNF-α ELISA kit (GIBCO BRL). The results of a representative experiment are shown in Exhibit 5. Less TNF-α was produced upon stimulation with 91K>A than with another non-91 analogue.

8. With regard to claim 6, a peptide analogue with a position 91 substitution was prepared as a pharmaceutical composition prior to January 1994. As shown in the bottom half of Exhibit 1, an experiment is presented in which alanine-substituted peptides, including 91K>A (89.5), were tested for their ability to induce EAE. For this test, the peptides were dissolved in PBS (a physiologically acceptable carrier or diluent) at 2 mg/ml and emulsified with an equal volume of CFA. Rats were then immunized subcutaneously in the hind foot pads with 0.1 ml of this emulsion and monitored for clinical signs of EAE. As shown, animals receiving 91K>A did not have symptoms of EAE.

9. The methods recited in claims 7-9 and 11 were conceived of prior to January 1994. As seen in Exhibit 6, which is an excerpt from a grant proposal submitted prior to January 1994, treatment of EAE, as a model system for MS, with peptide analogues was proposed. Moreover, the criteria set forth in the specification (page 6, lines 14-17), (a) competition for binding to MHC, (b) failure to cause proliferation of MBP-reactive T cell line, and (c) inhibit induction of EAE in rodents are parameters used to identify peptide analogues for potential therapeutic value. As shown in Exhibit 4, these same three criteria are set out as specific aims of the proposal (Aims 1a, 1b, and 2).

10. In subsequent experiments, the peptide analogue 91K→A was tested for its ability to treat EAE. Consistent with the teachings of the specification, especially page 8 and Example 8, EAE was induced by injection of MBP-reactive T cells or MBP 87-99 dissolved in PBS and emulsified with an equal volume of IFA supplemented with 4mg/ml heat killed *Mycobacterium tuberculosis* H37Ra in oil. Rats were immunized subcutaneously in the hind foot pads with 0.1 ml of the emulsion and were monitored daily for clinical signs of EAE. EAE was scored as follows: 0, clinically normal; 1, flaccid tail; 2, hind limb paralysis; and 3, front and hind limb paralysis. Animals were either co-immunized with MBP 91K→A at a 1:1 molar ratio. The peptide analogue 91K→A reduced both the incidence and severity of diseases.

In summary, upon review of our laboratory records and submitted proposals, of which the above-cited pages and Exhibits are representative, we have concluded that prior to

January 1994, we had conceived of synthesizing peptide analogues of human myelin basic protein containing a substitution at residue 91. Subsequently, MBP 87-99 with a position 91 substitution was synthesized and prepared in a pharmaceutically acceptable carrier or diluent prior to January, 1994. Also prior to this date, we had conceived of using position 91 substituted peptide analogues to treat multiple sclerosis. From this date until the filing of the application, we pursued in a diligent fashion experiments directed toward this goal of inducing EAE in rats and treating with the peptide analogue.

We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true and correct. Further, the undersigned understands that willful, false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Dated: 7/31/02 — LAWRENCE STEINMAN

Dated: July 11, 2000 — NICHOLAS LING

Dated: 7/11/00 — PAUL J. CONLON

EXHIBIT 1

		89	90	91	92	93	94	95	96	97	98	99	100	101
89-101		VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-89)	ALA	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-90)	VAL	ALA	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-91)	VAL	HIS	ALA	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-92)	VAL	HIS	PHE	ALA	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-93)	VAL	HIS	PHE	PHE	ALA	ASN	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-94)	VAL	HIS	PHE	PHE	LYS	ALA	ILE	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-95)	VAL	HIS	PHE	PHE	LYS	ASN	ALA	VAL	THR	PRO	ARG	THR	PRO
89-101	(ALA-96)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	ALA	THR	PRO	ARG	THR	PRO
89-101	(ALA-97)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	ALA	PRO	ARG	THR	PRO
89-101	(ALA-98)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	ALA	ARG	THR	PRO
89-101	(ALA-99)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ALA	THR	PRO
89-101	(ALA-100)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	ALA	PRO
89-101	(ALA-101)	VAL	HIS	PHE	PHE	LYS	ASN	ILE	VAL	THR	PRO	ARG	THR	ALA

EXHIBIT 2

Active induction 2mg/ml-mBS 1:1 with CFA 100µl/F.

group/day	13	14	15	16	First day of onset	Last day of onset	Duration
89.0	6/6	6/6	6/6	4/6	12	19	7
89.1	6/6	6/6	6/6	2/6	12	19	7
89.2	6/6	6/6	6/6	5/6	12	19	7
89.3	6/6	6/6	6/6	4/6	12	19	7
89.4	0/6	0/6	0/6	0/6	0	0	0
89.5	0/6	0/6	0/6	0/6	0	0	0
89.6	0/6	0/6	1/6	1/6	15	19	4
89.7	6/6	6/6	6/6	6/6	12	19	7
89.8	0/6	1/6	1/6	4/6	14	19	5
89.9	0/6	0/6	~~1/6~~ 0/6	0/6	0	0	0
89.10	0/6	0/6	0/6	0/6	0	0	0
89.11	4/6	4/6	4/6	3/6	12	19	7
89.12	6/6	6/6	5/6	4/6	12	19	7
89.13	3/6	3/6	3/6	0/6			

Signed

100-200mg of:	
87-99	VHFFKNIVTPRTP
87-99 Ala4	VHFAKNIVTPRTP
87-99 Ala5	VHFFANIVTPRTP
87-99 Ala6	VHFFKAIVTPRTP
87-99 Ala7	VHFFKNAVTPRTP
87-99 Ala8	VHFFKNIATPRTP
87-99 Ala9	VHFFKNIVAPRTP
87-99 Ala10	VHFFKNIVTARTP
100-200mg of:	
87-99 Ala5,6.	VHFFAAIVTPRTP
87-99 Gln6	VHFFKQIVTPRTP
87-99 Leu5	VHFFLNIVTPRTP
87-99 Val5	VHFFVNIVTPRTP
87-99 Arg5	VHFFRNIVTPRTP
87-99 Glu5	VHFFENIVTPRTP
87-99 Asn5	VHFFNNIVTPRTP
87-99 Tyr5	VHFFYNIVTPRTP
87-99 Ser5	VHFFSNIVTPRTP
87-99 Asp5	VHFFDNIVTPRTP
75-100 mg of:	
87-98	VHFFKNIVTPRT
87-97	VHFFKNIVTPR
87-96	VHFFKNIVTP
87-95	VHFFKNIVT
87-94	VHFFKNIV

EXHIBIT 3

D.2.4. Inhibition of TNFα Release. Recognition of the appropriate peptide by T cells is necessary, but not sufficient, as T cell clones with identical T cell receptors possess differing capacities to induce EAE. Encephalitogenicity correlates with the degree of production of TNFα (Powell et al., 1990). Sense and antisense primers and probes for TNFα and as a control, GM-CSF, will be synthesized and used in a PCR assay. This semiquantitative PCR assay involves measuring HPRT mRNA as an "internal standard" (O'Garra and Vieira, 1992). The PCR reaction involves adding 5 µl of each sample to each reaction tube and the following reagents are added: 5 µl of 10x buffer, 1 µl of 10 mM dNTP, 1 µl of each primer (10 OD/ml, sense and antisense), 37 µl of sterile water, and 0.2 ml of Taq Thermalase, 4 U/ml. The cycling conditions are 1 cycle at 92° C for 2 min, then 30 consecutive cycles

of 91° C for 19 sec, 59° C for 25 sec, and 72° C for 25 sec. A standard curve is constructed to determine relative amounts of mRNA expressed by each sample, from a "plateau region" to the "lowest detectable amount", as compared to a stock standard RNA control. Once a range has been established, one can further focus on the part of the curve that is of interest. Although absolute values will vary from run to run, due to different efficiencies of reverse transcription, PCR amplification, radioactive labeling and hybridization, the use of the standard curve with each reaction enables us to relate back to relative amounts of mRNA expression in a given sample, by reading its cpm value off the appropriate standard curve. RNA extraction and hybridization of amplified cDNA are according to the method of Panzara et al. (1992). Measurement of rat TNFα and IL-2 cytokines by bioassay will be done in a similar fashion as that reported by Powell et al. (1990).

In the next section, we will describe the details of the experiments designed to answer the Aim #2.

EXHIBIT 4

Subject

Binding Ex 2

Reversal of EAE

2502 adoptive EAE (day 0) 10×10^6 cells

day 2 2mg/ml Peptide in PBS I.P.)

day 4 "

day 6-7

Group	Peptide	
a	87.0	0/6
b	87.5	0/6
c	87.9	6/6
d		6/6

EXHIBIT 5

EXHIBIT 6

D.2.4. Prevention of EAE with antagonists. EAE is an autoimmune demyelinating disease induced by immunizing susceptible strains of rodents, rabbits, or non-human primates with MBP or with encephalitogenic peptide fragments. $CD4^+$ T cells are involved in this immune response (Zamvil et al., 1985), and genetic susceptibility in mice is based partly on the capacity of encephalitogenic peptides to bind to particular class II MHC molecules (Sakai et al., 1988 a & b; Wraith et al., 1989 a & b). Peptide analogs inhibiting the response of the MBP(89-101) specific cells *in vitro* will be analyzed *in vivo*. Conservatively then, only about 8-10 of the peptides (including Ala6) will be evaluated in this model. Groups containing 10 rats will be injected with MBP(89-101) alone or in the presence of the MBP antagonist subcutaneously along with the buffer control. Thus, 30 animals per peptide will be needed or 240-300 Lewis rats. The course of the disease will then be followed for approximately 3 weeks, and the extent of EAE determined as previously described (Sakai et al., 1988b). All experiments are run in a blinded fashion and those samples showing inhibition of EAE will also be evaluated histologically.

D.2.5. Treatment of ongoing EAE with antagonists. Those peptides which can inhibit the in vitro MBP specific T cell response and prevent the induction of EAE will then be tested in a therapeutic model to determine their efficacy. Groups of Lewis rats will be injected with MBP and at the first signs of paralysis (around days 9-10), they will be treated with either MBP peptide analog, an irrelevant peptide or buffer and the extent of EAE determined as previously described (Sakai et al., 1988b) over the next two weeks. All experiments are run in a blinded fashion and those samples showing inhibition of EAE will also be evaluated histologically. Conservatively then, only about 4-5 of the peptides will be evaluated in this model. Groups containing 10 rats will be injected when the symptoms of EAE first appear with either MBP peptide analogs (including Ala6), an irrelevant peptide or the buffer control. Thus, 30 animals per peptide will be needed or 150 Lewis rats.

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PATENT

I hereby certify that on the date specified below, this correspondence is being deposited with the United States Postal Service as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, DC 20231.

Date

Rozell A. Price

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Lawrence Steinman et al.
Application No.	:	08/953,937
Filed	:	October 20, 1997
For	:	METHODS FOR TREATMENT OF MULTIPLE SCLEROSIS USING PEPTIDE ANALOGUES AT POSITION 91 OF HUMAN MYELIN BASIC PROTEIN
Examiner	:	Jeffrey E. Russel
Art Unit	:	1654
Docket No.	:	690068.412C1
Date	:	January 20, 2001

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF AMITABH GAUR UNDER 37 C.F.R. § 1.131

Sir:

I, Amitabh Gaur declare that I have reviewed the Declaration Under 37 C.F.R. 1.131 executed by Lawrence Steinman, Nicholas Ling and Paul J. Conlon (copy attached). I declare that the substance of the present Declaration is identical to the Declaration signed by Lawrence Steinman, Nicholas Ling and Paul J. Conlon., with the exception that the terms we and our have been changed to I and my where appropriate.

I, Amitabh Gaur, do hereby declare that:

1. I am a coinventor of the subject matter in the above-identified patent application.

2. I, Amitabh Gaur, was employed at Neurocrine Biosciences, an assignee of the above-identified patent application from May 1994 to September 1999.

3. I have reviewed the Examiner's Office Action dated October 29, 1999 (Paper No. 32), especially the § 102 and § 103 rejections: claims 1, 3, 5, and 6 under 35 U.S.C. § 102(a) as being anticipated by Wucherpfennig et al.; claims 1-3, 5, and 6 under 35 U.S.C. § 102(a) as being anticipated by Vogt et al.; and claims 6-9 and 11 under 35 U.S.C. § 103 as being unpatentable over Sharma et al. in view of Wucherpfennig et al. or Vogt et al. In order to assist the Examiner's evaluation of the application, we have provided the following remarks showing that the present invention was conceived of prior to the publication of Wucherpfennig (the earlier date) and diligently reduced to practice, culminating in the filing of the subject application.

4. I have reviewed our laboratory records and submitted proposals, including the Exhibits submitted herewith, and readily conclude that the peptides recited in claims 1-3, 5 and 6 were conceived prior to January 1994 (the publication date of Wucherpfennig et al.) or August 11, 1994 (the publication date of Vogt et al.). Further, the invention of claims 1-3, and 5 were actually reduced to practice prior January 1994. In addition, the methods recited in claims 6-9 and 11 were conceived of prior to January 1994, and were diligently pursued up to the filing of the present application.

5. With regard to claims 1 and 2, Exhibit 1 is a laboratory page recorded prior to January 1994, showing conception of the sequences of MBP 87-99 analogues[1]. In particular, the analogue that has a K>A substitution at residue 91 is shown. (In Exhibit 1, this residue is labeled 93, consensus of the numbering of human MBP was reached later in time). In

[1] Dates originally recorded on the laboratory pages and grant proposals have been redacted. The time frame relative to the publication dates of the cited art is presented instead.

accordance with the teachings of the specification on pages 5-6 and Example 1, in these experiments, peptides of human myelin basic protein containing residues 87-99 were synthesized. The wild type peptide sequence and peptides with single alanine substitutions were synthesized by an automated procedure on a peptide synthesizer (model 5050; Milli Gen, Burlington, MA) using standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by HPLC; the structure was confirmed by amino acid analysis and mass spectroscopy. The sequence of the wild-type peptide, called 89.0, is shown at the top of Exhibit 1. The peptide analogue with a position 91 alanine substitution (position 93 in Exhibit 1) is labeled peptide 89.5. Thus, an analogue was made with a 91 substitution, in particular, from lysine to the non-conservative amino acid alanine. Furthermore, as shown in Exhibit 2, the 91K>A peptide was tested in a proliferation assay, such as the one described on page 7, lines 13-24 and Example 6 of the specification. The peptide analogue, 91K>A (labeled p89.5 in Exhibit 2) failed to stimulate proliferation of an MBP-reactive T cell line at a concentration range of 10-100 μM.

6. With regard to other peptide substitutions at position 91 as recited in claim 3, Exhibit 3, dated prior to January 1994, is a page from a laboratory notebook showing peptide analogues in which position 91 is altered to alanine, leucine, valine, arginine, glutamic acid, asparagine, tyrosine, serine and aspartic acid.

7. As recited in claim 5, position 91 peptide analogues reduce production of TNF-α from MBP-reactive T cells. This concept was proposed in an application which was submitted to a granting agency prior to January 1994. In this proposal, one aim was specifically directed toward determining cytokine profiles of T cell clones that are reactive to MBP after incubation with native or mutated MBP peptides (Exhibit 4). Experiments were subsequently performed to examine the expression levels of TNF-α upon exposure to position 91 analogues in particular. Briefly, as similarly described in Example 11, draining lymph node cells from rats co-immunized with MBP 87-99 and MBP 87-99 [91K→A] were stimulated *in vitro* (10^7 cells/ml) with different concentration of peptides. After 12, 24, 48, and 72 hr of stimulation, supernatants were collected for cytokine detection. TNF-α expression levels were determined after 8 hr by a rat TNF-α ELISA kit (GIBCO BRL). The results of a representative experiment are shown in

Exhibit 5. Less TNF-α was produced upon stimulation with 91K>A than with another non-91 analogue.

8. With regard to claim 6, a peptide analogue with a position 91 substitution was prepared as a pharmaceutical composition prior to January 1994. As shown in the bottom half of Exhibit 1, an experiment is presented in which alanine-substituted peptides, including 91K>A (89.5), were tested for their ability to induce EAE. For this test, the peptides were dissolved in PBS (a physiologically acceptable carrier or diluent) at 2 mg/ml and emulsified with an equal volume of CFA. Rats were then immunized subcutaneously in the hind foot pads with 0.1 ml of this emulsion and monitored for clinical signs of EAE. As shown, animals receiving 91K>A did not have symptoms of EAE.

9. The methods recited in claims 7-9 and 11 were conceived of prior to January 1994. As seen in Exhibit 6, which is an excerpt from a grant proposal submitted prior to January 1994, treatment of EAE, as a model system for MS, with peptide analogues was proposed. Moreover, the criteria set forth in the specification (page 6, lines 14-17), (a) competition for binding to MHC, (b) failure to cause proliferation of MBP-reactive T cell line, and (c) inhibit induction of EAE in rodents are parameters used to identify peptide analogues for potential therapeutic value. As shown in Exhibit 4, these same three criteria are set out as specific aims of the proposal (Aims 1a, 1b, and 2).

10. In subsequent experiments, the peptide analogue 91K→A was tested for its ability to treat EAE. Consistent with the teachings of the specification, especially page 8 and Example 8, EAE was induced by injection of MBP-reactive T cells or MBP 87-99 dissolved in PBS and emulsified with an equal volume of IFA supplemented with 4mg/ml heat killed *Mycobacterium tuberculosis* H37Ra in oil. Rats were immunized subcutaneously in the hind foot pads with 0.1 ml of the emulsion and were monitored daily for clinical signs of EAE. EAE was scored as follows: 0, clinically normal; 1, flaccid tail; 2, hind limb paralysis; and 3, front and hind limb paralysis. Animals were either co-immunized with MBP 91K→A at a 1:1 molar ratio. The peptide analogue 91K→A reduced both the incidence and severity of diseases.

In summary, while my employment with Neurocrine did not begin until May 1994, on information and belief that the notebook records and discussions with my co-inventors are believed to be true and correct and further upon review of our laboratory records and submitted proposals, of which the above-cited pages and Exhibits are representative, I have concluded that prior to January 1994, we had conceived of synthesizing peptide analogues of human myelin basic protein containing a substitution at residue 91. In addition, on information and belief that the notebook records and discussions with my co-inventors are believed to be true and correct, I conclude that MBP 87-99 with a position 91 substitution was synthesized and prepared in a pharmaceutically acceptable carrier or diluent prior to January, 1994 and that prior to this date, we had conceived of using position 91 substituted peptide analogues to treat multiple sclerosis. Further, it is my belief that at least prior to January 1994 until the filing of the application, we diligently pursued experiments directed toward the goal of inducing EAE in rats and treating with the peptide analogue to model treatment of multiple sclerosis.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and correct. Further, the undersigned understands that willful, false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Dated: March 19, 2001

Amitabh Gaur